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STUDIES ON HEMORRHAGE

MARY ADELIA BENNETT

A THESIS

IN PHYSIOLOGICAL CHEMISTRY
PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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SOME CHANGES IN THE ACID-BASE EQUILIBRIUM OF THE BLOOD CAUSED BY HEMORRHAGE

A COMPARISON OF THE pH OF SERUM AND PLASMA OF DOG BLOOD

A NOTE ON CULLEN'S COLORIMETRIC METHOD FOR THE DETERMINATION OF THE pH OF BLOOD PLASMA

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SOME CHANGES IN THE ACID-BASE EQUILIBRIUM OF THE BLOOD CAUSED BY HEMORRHAGE.*

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INTRODUCTION.

Many investigations have been made upon the effect of hemorrhage on the acid-base equilibrium of the body. Most of these experiments deal with variations in the alkaline reserve of the blood. The results obtained have been complicated in several cases, either by anesthetics or by struggling. Few observations have been made on the reaction of the blood after hemorrhage. With a view of obtaining more data to permit better correlation of some of the factors involved in the acid-base equilibrium, the effect of hemorrhage on the pH, CO_2 content, and CO_2 tension of arterial blood was studied.

Milroy (1917) studied the effect of large hemorrhages upon the reserve alkali in the blood of cats and dogs and found a decrease which reached a minimum value in 15 minutes. Since then Cannon (1918), Penfield (1919), Buell (1919), Gesell (1919), Tatum (1920), Evans (1921), and Henderson and Haggard (1922), have also found a decrease in alkaline reserve or CO_2 capacity of blood after bleeding.

Miss Buell (1919) found in pigs, after hemorrhages amounting to 1.3 per cent of the body weight, a minimum alkaline reserve occurring in half an hour, with a return to normal in about 5 hours. Tatum (1920) observed that hemorrhages in rabbits produced a fall in alkaline reserve which was followed on the next day by values above normal. Evans (1921) found a similar swing from low to high values. The high alkaline reserve persisted until the red cell regeneration seemed to be complete. Barr and Peters (1920–21) and Means, Bock, and Woodwell (1921) have observed high alkaline reserves in some anemic patients, but not in all the cases studied.

* A brief report of this paper is given in *Proc. Soc. Exp. Biol. and Med.*, 1925, xxiii, 114.

Von Endres (1922) found an increased CO_2 tension after hemorrhage in two human subjects.

Evans (1921) studied the pH of the blood of two cats under urethane after hemorrhage by the Dale and Evans colorimetric method. In one case he found a decrease of 0.14 pH in 35 minutes following the bleeding; in the other case, there was an increase of 0.13 pH 18 minutes after the hemorrhage.

Methods.

Dogs were used throughout the experiments. Dog 2 had had an operation for an Eck fistula several months previous to the

TABLE I.

Dog 1, Female, Weight 13 Kilos, on Mixed Diet.

Date.	Time.	Hematocrit reading.	Oxalated arterial blood plasma.				Remarks.
			Electrometric pH.*	CO_2 content.	CO_2 tension.	Combined CO_2 at normal pH (alkaline reserve).	
1925		*per cent*		*mM*	*mm. Hg*	*mM*	
June 13	11.00 a.m.	39	7.31	22.0	41.9	20.7	
" 25	10.30 "	40	7.31	20.1	38.3	18.9	
July 8	2.00 p.m.	40	7.30	21.0	41.0	19.5	
" 9	10.00 a.m.						10.00–10.30 a.m. bled 300 cc. from jugular vein.
" 9	10.45 "	40					
" 9	2.00 p.m.	35	7.22	19.2	44.4	15.0	
" 10	2.30 "	28	7.37	19.2	32.0	19.9	Dog killed.

* Electrometric pH determinations not done in duplicate.

hemorrhage experiments, but as it appeared to be normal and gave results similar to those of the other dogs, there was probably no complication from that source.

The blood for analysis was taken from the left ventricle or from the femoral artery. No anesthetic was given and the animals did not struggle. The heavy bleedings ranging from one-fourth to one-half the total blood volume calculated as 7 per cent of the body weight were made from the jugular vein in the first two

and last two experiments and from the left ventricle in the others. In the first two experiments, the blood for analysis was drawn over mercury into a tonometer containing dried potassium oxalate and sodium fluoride, as suggested by Evans (1922), sufficient to make

TABLE II.

Dog 2, Female, with Eck Fistula, Weight 8 Kilos, on Mixed Diet.

Date.	Time.	Hematocrit reading.	Oxalated arterial blood plasma.				Remarks.
			Electrometric pH.*	CO_2 content.	CO_2 tension.	Combined CO_2 at normal pH (alkaline reserve).	
1924		*per cent*		*mM*	*mm. Hg*	*mM*	
May 10	10.30 a.m.		7.34	21.7	38.7	20.3	
" 15	10.00 "	34	7.37	22.4	37.5	21.8	
" 16	10.15 "						10.15–10.30 a.m. bled 200 cc. from jugular vein.
" 16	10.45 "	31	7.29	16.9	33.6	14.0	
" 16	3.15 p.m.	27		16.6			
" 17	12.30 "	22	7.45	23.7	33.2	25.0	
" 19	2.45 "	20	7.40	20.2	31.7	20.0	
" 26	3.00 "	21	7.35	20.6	36.0	19.5	
" 27	10.30 a.m.						10.30–11.00 a.m. bled 215 cc. from jugular vein.
" 27	11.15 "	21	7.36	19.5	33.4	18.5	
" 27	4.00 p.m.		7.25	19.4	42.3	15.2	
" 28	2.00 "	14		20.3			
" 31	3.00 "	15	7.36	21.9	37.4	20.9	
July 16	10.30 a.m.	24	7.26	19.2	40.7	15.3	

* Electrometric pH determinations not done in duplicate.

a 0.3 per cent and a 0.1 per cent solution respectively. The blood was then mixed well and transferred to centrifuge tubes under oil. The tubes were completely filled and closed by a rubber stopper, or the blood was overlaid with low-melting paraffin as suggested by Hastings and Sendroy (1924). After centrifuging

at high speed for about 15 minutes, the plasma was drawn off into a tonometer over mercury and was ready for analysis. This procedure was the same as that described by Austin, Cullen, Hastings,

TABLE III.

Dog 3, Female, Weight 22 Kilos, on Milk Diet.

Date.	Time.	Hematocrit reading.	Arterial blood serum.				Remarks.
			Electrometric pH.	CO_2 content.	CO_2 tension.	Combined CO_2 at normal pH (alkaline reserve).	
1925		*per cent*		*mM*	*mm. Hg*	*mM*	
Jan. 19	12.00 m.	37	7.33	24.6	44.9	23.3	
" 21	10.00 a.m.						10.00–10.10 a.m. bled 450 cc. from heart.
" 21	10.40 "	32	7.31	18.7	35.7	17.0	
" 21	2.30 p.m.	25	7.26	21.4	45.5	18.1	
" 22	1.30 "	21	7.38	23.7	38.8	23.9	
" 24	10.00 a.m.	19	7.34	22.0	39.4	21.0	
" 27	10.00 "	20	7.33	23.2	42.3	21.9	
" 28	10.00 "						10.00–10.10 a.m. bled 400 cc. from heart.
" 28	10.40 "	19	7.32	18.5	34.5	17.1	
" 28	2.30 p.m.	12	7.31	21.8	41.5	20.0	
" 29	12.00 m.	12	7.39	23.4	37.4	24.0	
Feb. 5	11.00 a.m.	12	7.34	21.4	38.2	20.5	
" 18	10.00 "	20	7.33	19.7	36.0	18.6	
" 18	10.00 "						10.00–10.10 a.m. bled 400 cc. from heart.
" 18	10.45 "			15.4			
" 18	3.00 p.m.	10	7.32	17.4	32.5	16.0	
" 19	10.00 a.m.	11	7.38	22.2	35.5	22.5	

McLean, Peters, and Van Slyke (1922). In the last three experiments where serum was used, the blood was collected directly under oil, and centrifuged after clotting had occurred.

The pH of the blood was determined by the electrometric

method and Cullen's colorimetric method (1922). Electrometric pH determinations were made at 38°C. A portion of plasma or serum in a small Clark-Cullen electrode vessel was allowed to come into equilibrium with a carbon dioxide and hydrogen mixture which had approximately the carbon dioxide tension of the blood, as suggested by Cullen (1922). The serum or plasma was replaced by fresh portions until constant E.M.F. readings were obtained with the same gas mixture. Standard apparatus, with

TABLE IV.

Dog 4, Female, Weight 13.9 Kilos, Fasted.

Date.	Time.	Hematocrit reading.	Arterial blood serum.				Remarks.
			Electrometric pH.	CO_2 content.	CO_2 tension.	Combined CO_2 at normal pH (alkaline reserve).	
1925		*per cent*		*mM*	*mm. Hg*	*mM*	
Feb. 11	10.00 a.m.	34	7.34	19.3	34.5	18.3	
" 11	10.00 "						10.00–10.10 a.m. bled 425 cc. from heart.
" 11	2.30 p.m.	25	7.32	21.5	40.0	19.9	Venous blood.
" 12	10.00 a.m.	20	7.35	18.1	31.6	17.5	
" 25	10.30 "	23	7.34	19.5	34.8	18.5	
" 25	10.30 "						10.30–10.40 a.m. bled 400 cc. from heart.
" 25	3.30 p.m.	26	7.32	18.2	33.9	16.7	
" 26	10.45 a.m.	23	7.37	19.7	32.9	19.4	

a saturated calomel electrode, a saturated KCl bridge, a type K Leeds and Northrup potentiometer and a galvanometer, was used. The determinations were standardized against 0.1 N HCl by calculating "e" for the entire system assuming a pH of 1.080 at 38°C. (Cullen, Keeler, and Robinson, 1925). A phosphate mixture with pH 7.4 was standardized against 0.1 N HCl and used before and after each series of plasma or serum readings. Duplicate determinations were made except where designated.

The colorimetric pH determinations were carried out using Cullen's method (1922). The readings were made at 20°C. The standard phosphate buffer stock solutions were tested electrometrically. New standards were prepared from them every week and all the solutions were kept in the ice box while not in use. Under these conditions the color standards did not change

TABLE V.

Hemorrhage and Gelatin Saline Injection. Dog 5, Female, Weight 11.4 Kilos, Fasted.

Date.	Time.	Hematocrit reading.	Arterial blood serum (defibrinated).				Remarks.
			Electrometric pH.	CO_2 content.	CO_2 tension.	Combined CO_2 at normal pH (alkaline reserve).	
1925		*per cent*		*mM*	*mm. Hg*	*mM*	
Feb. 21	10.30 a.m.	31	7.35	19.6	34.3	18.6	
" 23	10.30 "						10.30–10.40 a.m. bled 400 cc. from heart. 10.40–11.20 a.m. injected 400 cc. of 0.9 per cent sodium chloride solution containing 6 per cent gelatin.
" 23	11.25 "	12	7.26	13.8	29.2	10.0	
" 23	4.00 p.m.	14	7.44	12.2	17.4	14.2	
" 24	11.00 a.m.	11	7.31	14.6	27.9	12.2	

during a week. The data of the colorimetric determinations are reported in the following papers.

Carbon dioxide determinations were made on the serum or plasma using Van Slyke's volumetric apparatus (Van Slyke and Stadie, 1921).

The arterial CO_2 tensions were calculated from the pH and total CO_2 values by means of a rearrangement of Hasselbalch's equation used by Austin, Cullen, Hastings, McLean, Peters, and Van Slyke (1922).

$$p = \frac{CO_2 \text{ mM}}{0.0587\ \alpha\ (10^{pH - pK'} + 1)}$$

$\alpha = 0.935 \times 0.555$, the solubility coefficient for CO_2 in serum at 38°C. (Van Slyke, Wu, and McLean, 1923). $pK' = 6.1$ for plasma or serum (Van Slyke, Austin, and Cullen, 1922; Cullen, Keeler, and Robinson, 1925).

The combined CO_2 at normal pH (alkaline reserve) was calculated according to Van Slyke, Austin and Cullen (1922). The normal pH was interpolated on the pH:$BHCO_3$ graph obtained after hemorrhage, and the combined CO_2 read off at that point. In order to determine the combined CO_2 in the blood as drawn, the free CO_2 was calculated from the following formula for plasma or serum (Austin, Cullen, Hastings, McLean, Peters, and Van Slyke, 1922).

$$\text{mM } H_2CO_3 = \frac{\alpha\ p}{760 \times 0.0224}$$

p = CO_2 tension.

$\alpha = 0.935 \times 0.555$ (Van Slyke, Wu, and McLean, 1923).

With the total CO_2 known, the combined CO_2 may be calculated.

$$\text{mM total } CO_2 - \text{mM } H_2CO_3 = \text{mM } BHCO_3.$$

In order to plot such a graph as the one previously mentioned, pH against combined CO_2, with data available at only one point, a line was drawn through this point having an average slope determined by experiments on Dogs A and B to be reported later in this paper. In these experiments two points on the CO_2 absorption curves were determined by analysis of equilibrated blood. This slope was found to be practically the same as that used by Van Slyke, Austin, and Cullen (1922) in the curve plotted for an ether anesthesia experiment in their Fig. 1.

The hematocrit determinations were made on oxalated blood in a small Daland hematocrit.

There are included, in this report, two preliminary experiments, A and B, carried out by Dr. D. W. Wilson in 1922 in which the pH was determined by CO_2 absorption curves of arterial blood. Two portions of oxalated blood were equilibrated at

TABLE VI.

Dog A, Male, Weight 20.4 Kilos, on Mixed Diet.

Date.	Time.	Hematocrit reading.	Arterial whole blood.								Remarks.
			Equilibrated blood.				Blood as drawn.			Combined CO_2 at normal pH (alkaline reserve).	
			a.		*b.*						
			CO_2 content.	CO_2 tension.	CO_2 content.	CO_2 tension.	CO_2 content.	Calculated CO_2 tension.	Calculated pH.		
1922		*per cent*	*mM*	*mm. Hg*	*mM*	*mm. Hg*	*mM*	*mm. Hg*		*mM*	
Mar. 6	9.50 a.m.	41	17.1	37.8	13.7	22.8	16.7	35.0	7.35	15.9	
" 6	1.10 p.m.										1.10–1.45 p.m. bled 500 cc. from jugular vein.
" 6	1.50 "	42	14.4	37.7	11.0	23.0	12.9	32.1	7.30	10.7	
" 6	3.00 "	34	14.8	37.5			13.8	33.9	7.28	11.0	
" 7	11.00 a.m.	33	17.0	36.3	14.2	22.3	16.2	32.0	7.36	15.9	
" 8	10.00 "	35	17.3	33.3	15.1	22.3	17.1	31.8	7.40	17.0	
" 9	1.25 p.m.	31	18.8	33.1	16.4	22.8	19.1	36.0	7.40	18.5	
" 10	9.30 a.m.	32	18.2	35.4			18.3	35.5	7.37	17.5	
" 10	11.10 "										11.10–11.25 a.m. bled 265 cc. from jugular vein.
" 10	11.25 "	30									
" 10	12.25 p.m.	29	18.6	35.5	15.5	22.0	18.1	34.0	7.40	18.0	
" 11	11.00 a.m.	25	19.5	35.2	16.5	21.6	18.4	30.5	7.45	19.2	
" 13	3.30 p.m.	26	18.4	35.9	14.9	23.0	18.0	34.5	7.38	17.3	

38°C., one with about 5 per cent and the other with about 3 per cent CO_2 in air, and the total CO_2 content determined in each. The total CO_2 of the blood as drawn was also determined.

The pH values of the two equilibrated blood samples calculated by means of Hasselbalch's equation were plotted as abscissæ against the CO_2 content as ordinates. By interpolating on these lines the CO_2 in air content of the blood as drawn, the pH of the blood could be read.

Hasselbalch's equation requires values for the combined and free CO_2 of the blood in order to calculate the pH. The free CO_2 was calculated by means of Peters, Bulger, and Eisenman's (1923) modification of the simple formula involving the solubility coefficient (α) and CO_2 tension (p) whereby the difference in solubility of CO_2 in corpuscles and plasma is taken into account. The values for α determined by Van Slyke, Wu, and McLean (1923) were used; namely, 0.935 for serum, and 0.71 for cells.

The formula is as follows:

$$\text{mM } H_2CO_3 = \frac{p \times 0.555}{760 \times 0.0224} (0.935\ (1 - c) + 0.71\ c).$$

p = CO_2 tension in mm. Hg.

c = hematocrit reading.

With the total CO_2 known the combined CO_2 may be calculated.

$$\text{mM total } CO_2 - \text{mM } H_2CO_3 = \text{mM } BHCO_3.$$

The values for free and combined CO_2 were substituted in Hasselbalch's formula

$$pH = pK' + \log \frac{BHCO_3}{H_2CO_3}$$

using $pK' = 6.1$ and correcting the calculated pH for the variations in corpuscle concentration as suggested by Peters, Bulger, and Eisenman (1923) using their Chart 4.

The CO_2 tension of the blood as drawn was estimated by interpolation of the CO_2 content on a CO_2 content:tension curve plotted from the two equilibrated blood samples.

The combined CO_2 at normal pH (alkaline reserve) was calcu-

TABLE VII.

Dog B, Female, Weight 13.6 Kilos, on Mixed Diet.

Date.	Time.	Hematocrit reading.	Arterial whole blood.								Remarks.
			Equilibrated blood.				Blood as drawn.			Combined CO_2 at normal pH (alkaline reserve).	
			a.		*b.*						
			CO_2 content.	CO_2 tension.	CO_2 content.	CO_2 tension.	CO_2 content.	Calculated CO_2 tension.	Calculated pH.		
1922		*per cent*	*mM*	*mm. Hg*	*mM*	*mm. Hg*	*mM*	*mm. Hg*		*mM*	
Apr. 5	2.30 p.m.	43	19.2	40.4	15.4	22.8	18.5	36.5	7.40	17.2	
" 6		41	20.2	40.0	15.1	22.9	18.3	32.3	7.44	18.6	
" 7	11.40 a.m.										11.40 a.m.–12.00 m. bled 415 cc. from jugular vein.
" 7	2.00 p.m.	42	18.8	39.4	14.5	22.4	18.1	37.2	7.39	16.8	
" 8	10.00 a.m.	31	22.0	35.6	18.6	23.1	21.8	34.5	7.52	22.8	
" 10		29	20.6	35.1	17.0	21.9	20.7	36.0	7 43	20.0	
" 11	11.10 "										11 10–11 43 a.m bled 390 cc. from jugular vein
" 11	2.30 p.m.	33	17.9	34.1	14.4	22.5	16.8	31.0	7.42	16.5	
" 12	2.15 "	18	25.3	35.7	21.9	21.6	25.0	34.9	7 50	26.0	
" 13	2.40 "	19	22.2	35 5	19.5	21.3	22.2	35.0	7.44	21.8	
" 14	9.45 a.m.	18	21.7	35.2	19.1	23.1	21.6	35.1	7.44	21.0	
" 18	2.00 p.m.	19	18 7	35.5	16.0	22.4	18.1	33.9	7.39	17.0	

lated according to the method previously described. In these experiments, however, two points were known on the $BHCO_3$:pH curve, since the blood was equilibrated at two different CO_2 tensions.

EXPERIMENTAL.

The blood after hemorrhage showed the following changes in arterial pH, alkaline reserve, total CO_2 content, CO_2 tension, and hematocrit readings.

About half an hour after the hemorrhage, the pH was usually lower than normal. The decrease ranged from 0.01 pH to 0.08 pH. Still lower values were observed 4 or 5 hours later when the total decrease noted varied from 0.02 pH to 0.12 pH.

On the following day, however, the blood had become more alkaline and the pH was 0.01 to 0.11 above the normal values. At this time the hematocrit readings had reached a minimum showing that the blood volume was restored. In a few days the pH was back to normal. In the case of Dog 3 (Table III) the pH was normal 3 days after the first hemorrhage.

The sudden fall in alkaline reserve of the blood after hemorrhage, found by other investigators, was also observed in the present experiments. In about half an hour after the bleeding the alkaline reserve had fallen from 1.0 to 7.8 mM. 4 hours after hemorrhage the values were higher in most instances.

Within 24 hours the alkaline reserve had risen still further but was usually not above normal. In a few days the values had returned practically to normal or slightly below.

The CO_2 tension fell in half an hour after the hemorrhage, the fall ranging from 4 to 9 mm. of Hg. In 4 hours, the CO_2 tension had risen. The values obtained varied from 11.6 mm. of Hg below to 4.9 mm. of Hg above normal. The next day the CO_2 tension was always low, 1.5 to 8.8 mm. of Hg below normal. It came back to normal, or nearly normal, in a few days.

The hematocrit readings fell rapidly, slowly, or not at all in $\frac{1}{2}$ hour after hemorrhage. The drop observed was from 0 to 5 per cent. The hematocrit readings were falling 4 hours after the hemorrhage in all cases. The inflow of tissue fluid was gradual.

On the day following the hemorrhage, the hematocrit readings showed minimum values, signifying that the blood volume had

been restored. The lowest hematocrit reading obtained was 10 per cent, less than one-third of the normal value, yet the dog lived and seemed to be in good condition.

The normal hematocrit reading for Dog 2 (Table II) was 34; after two bleedings it fell to 14 and in 49 days was found to be only 24.

In the case of Dog 5 (Table V) where gelatin solution was injected to restore the original blood volume after hemorrhage, the pH was 0.09 above normal in 5 hours, although it fell 0.09 pH below normal immediately after the injection. This also suggests that the high pH values are associated with restoration of blood volume.

DISCUSSION.

It is important to consider how the data obtained from the above experiments may be correlated with those from other sources and with various theories concerning the response of the body to hemorrhage.

Immediately following a hemorrhage of the magnitude of those produced in our experiments—one-fourth to one-half the total blood volume—there is a fall in blood pressure due to the diminished volume of circulating fluid. As a result of the low blood pressure, low blood volume, and constriction of the peripheral vessels, there is a considerable decrease in the rate of blood flow (Cannon, Fraser, and Hooper, 1918; Robertson and Bock, 1919; Gesell, 1923, 1925). To restore an adequate circulation the pulse rate increases and tissue fluid passes into the blood.

In the present experiments hematocrit readings indicate the rate and extent of dilution of the remaining blood if it may be assumed that no great stores of corpuscles were poured into the circulation. No increase in blood volume occurred in some experiments, while in others the first blood sample taken after hemorrhage showed a greater or less dilution of the corpuscles. The hematocrit readings of blood taken 4 hours after a hemorrhage were always low and minimum values were obtained from blood taken the next day. It may be assumed, therefore, that the circulating blood volume had reached a maximum within 24 hours. This agrees with the observations of Carrier, Lee, and Whipple (1922).

The reduction of the circulation rate of blood which has been found to occur after large hemorrhages leads to an insufficient transport of oxygen to the tissues and to an increased respiration (Cannon, 1918; Henderson and Haggard, 1922; Gesell, 1923). The generally accepted opinion has been that this lack of oxygen results in the formation of lactic acid (Fletcher and Hopkins, 1902, 1907; McGinty and Gesell, 1925).

Some years ago Haggard and Henderson (1920) proposed a unique hypothesis to explain the decrease in alkaline reserve after hemorrhage. Associating the diminished CO_2 content and capacity of blood with the increased ventilation of the lungs, they suggested that the sequence is quite different from that commonly believed to occur. They consider that oxygen-want, due to the diminution of hemoglobin after hemorrhage causes an increased activity of the respiratory center and overaeration of the blood. The blood becomes more alkaline than normal and to compensate for this, alkali leaves the blood and enters the tissues. Thus the low CO_2 capacity is due to diffusion of sodium bicarbonate from the blood, rather than to its neutralization by acids diffusing from the tissues into the blood.

Such an hypothesis fails to explain adequately the data obtained from the present experiments. The blood is slightly more acid than normal a half hour after hemorrhage and the pH continues to diminish for several hours. Even if a short period of overventilation should occur immediately after hemorrhage[1] it seems impossible that excessive ventilation should lead to so great a diffusion of base from the blood as to cause a diminished pH. Overventilation should certainly maintain an increased pH of the blood. Therefore, it may be concluded from the data available that the ventilation, though above normal, is inadequate to remove enough CO_2 from the blood to maintain a normal pH when acid metabolic products pour in.

On the day following the hemorrhage, the blood is more alkaline than normal with a CO_2 capacity which has risen to normal. This

[1] Gesell and Hertzman state in a preliminary report (1925) that the blood actually becomes more alkaline immediately after hemorrhage but a rapid swing to the acid side of normal occurs suddenly. The abstract of the report does not permit a comparison of the time relations with the experiments in this paper.

is obviously contrary to the prediction of the theory of Haggard and Henderson. It appears, therefore, that a more satisfactory explanation of the present findings lies in the older conception that the lowered pH and CO_2 capacity are due to the passage into the blood of acids formed by inadequate oxidation during the period immediately following hemorrhage. As the transport of oxygen improves with the restoration of blood volume and circulation rate, the acid products disappear and the CO_2 capacity of the blood increases.

Milroy (1917) has suggested that the lowered reserve alkali found directly after hemorrhage is due to a rapid dilution of the blood with tissue fluids of lower bicarbonate content. The present experiments do not confirm his conclusions because the first sample of blood after hemorrhage often showed no dilution, while the alkaline reserve was lowered considerably. Moreover, the alkaline reserve may fall to a minimum and start to rise while the hematocrit readings are still falling. It seems, therefore, that the low alkaline reserve is not due merely to the dilution of the blood with tissue fluids.

Evans (1921), experimenting on rabbits, found that following hemorrhage the alkaline reserve first fell, then rose from 5 to $12\frac{1}{2}$ hours later, remaining above normal for many days until the hematocrit reading was restored to normal. These results differ considerably from those obtained in the present investigations on dogs. Using a colorimetric method, Evans studied the pH of anesthetized cats and reports two experiments of which one shows an increased pH of blood after hemorrhage. The unusually low "normal" value renders the observation of doubtful significance.

The slow circulation rate observed after hemorrhage by various investigators is associated with increased respiration. As oxygen transport is below normal at this time, there is probably a lack of oxygen in the respiratory center, which results in the increased respiration observed. Winterstein in 1921 suggested that the respiration may be regulated not only by the hydrogen ion concentration of the blood, but also by the hydrogen ion concentration developed in the respiratory center itself due to its own metabolism. According to this view, the hydrogen ion concentration produced in oxygen-want and similar conditions, by acid meta-

bolic products formed in the center, is the important factor in respiratory regulation and may lead to more alkaline blood by overaeration. Gesell (1923) has extended the conception and has obtained data with manganese dioxide electrodes and anesthetized animals which indicate that there may be no correlation at times between the hydrogen ion concentration of the blood and the activity of the center (Gesell and Hertzman, 1925). He believes that the metabolism of the center, together with the efficiency of removal of acid metabolic products, carbon dioxide and lactic acid, may be considered the points of fundamental importance.

A few aspects of the present experiments may be considered from this point of view. The increased lung ventilation found after hemorrhage caused a diminished CO_2 tension, but not an overaeration, since the pH of the arterial blood decreased. The lowered alkaline reserve indicates an extensive acid production throughout the body as early as $\frac{1}{2}$ hour after the hemorrhage. The increased respiration may therefore be due to lactic acid production in the center caused by inadequate oxygen transport. That nerve tissue may form lactic acid is demonstrated by the investigations of McGinty and Gesell (1925).

The diminished hematocrit reading and the increasing alkaline reserve, which occur in 4 hours or more after hemorrhage, give evidence of improvement in the circulation. If the rise in alkaline reserve is due to oxidative removal of lactic acid in tissues, such as muscles, this same process may be presumed to occur in the respiratory center. This would lead to an increase in the alkalinity of the center, a decrease in respiration, and an increase in arterial CO_2 tension. But it is difficult to understand how the center can become so alkaline as to cause a decrease in respiration which would result in an accumulation of carbon dioxide sufficient to decrease the pH of the blood still further. CO_2 tensions above normal have sometimes been found. It seems difficult to account for the maintenance of such a low pH of the blood by the presence of so high a concentration of easily diffusible acid which may diffuse into the respiratory center, unless the activity of the respiratory center has been altered by factors other than the hydrogen ion concentration within the center.

On the day following the hemorrhage the situation is quite

different. The maximum dilution of the blood has occurred with the accompanying increased rate of blood flow. Data obtained from the acacia experiments of Gesell and his coworkers, would suggest that the "nutrient flow" (blood flow × hemoglobin) may even be unexpectedly high and perhaps not far from normal. Under these conditions the lung ventilation is sufficient to maintain a pH of the blood above normal and a low CO_2 tension. This demands overactivity of the respiratory center. A simple explanation would be that overactivity is due to an excess of acid in the center itself. This might result from decreased oxygen and CO_2 transport due to the low concentration of hemoglobin in the blood. But before accepting such an hypothesis, the decreased transport of oxygen and CO_2 should be demonstrated in view of the following considerations. That normal supplies of oxygen are available in anemia has been shown by Lundsgaard (1919). He found that patients with anemia could use their reserve oxygen. Until complete reduction of the oxyhemoglobin to hemoglobin failed to furnish sufficient oxygen to the tissues, there was no compensatory increase in the rate of circulation in the resting organism. As the alkaline reserve is normal in the experiment described in the present paper (sometimes above) it is obvious that oxygen is furnished to the tissues, at least to the muscles, in quantities sufficient to keep the production of lactic acid within normal limits. This indicates that the oxygen transport is maintained within normal limits. The isohydric change of oxyhemoglobin to hemoglobin accounts for the carrying of 75 per cent or more of the carbon dioxide from the tissues. Therefore if normal quantities of oxygen are being furnished to the tissues by a rapid flow of diluted blood, the normal acid transport can hardly be seriously affected. Of course, the respiratory center may be more sensitive than other tissues to a small accumulation of acid due to a slight oxygen-want or slightly deficient acid transport. But any explanation which may be given to account for overactivity of the center on the day following a hemorrhage must take into account the fact that without any appreciable change in concentration of red cells, an adjustment in respiration occurs within a few days so that the pH of the blood again returns to normal.

The conclusion may perhaps be drawn from the above dis-

cussion that variations in the hydrogen ion concentration of the blood cannot be used alone to prove that inverse variations in hydrogen ion concentration have taken place in the respiratory center.

SUMMARY.

Blood was studied at various intervals after large hemorrhages in dogs.

After hemorrhage there was a rapid acid production as indicated by a fall in pH and alkaline reserve. The alkaline reserve started to rise quickly and was normal on the day following the hemorrhage. At this time the pH had swung to the alkaline side of normal. It returned to normal after several days.

The observations are discussed from the point of view of several recent theories concerning the regulation of blood alkali and the activity of the respiratory center.

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BIBLIOGRAPHY.

Austin, J. H., Cullen, G. E., Hastings, A. B., McLean, F. C., Peters, J. P., and Van Slyke, D. D., *J. Biol. Chem.*, 1922, liv, 121.

Barr, D. P., and Peters, J. P., Jr., *J. Biol. Chem.*, 1920–21, xlv, 571.

Buell, M. V., *J. Biol. Chem.*, 1919, xl, 29.

Cannon, W. B., *J. Am. Med. Assn.*, 1918, lxx, 531.

Cannon, W. B., Fraser, J., and Hooper, A. N., *J. Am. Med. Assn.*, 1918, lxx, 526.

Carrier, E. B., Lee, F. W., and Whipple, G. H., *Am. J. Physiol.*, 1922, lxi, 138.

Cullen, G. E., *J. Biol. Chem.*, 1922, lii, 501.

Cullen, G. E., Keeler, H. R., and Robinson, H. W., *J. Biol. Chem.*, 1925, lxvi, 301.

von Endres, G., *Biochem. Z.*, 1922, cxxxii, 220.

Evans, C. L., *Brit. J. Exp. Path.*, 1921, ii, 105.

Evans, C. L., *J. Physiol.*, 1922, lvi, 146.

Fletcher, W. M., and Hopkins, F. G., *J. Physiol.*, 1902, xxviii, 354, 474; 1907, xxxv, 247.

Gesell, R., *Am. J. Physiol.*, 1919, xlvii, 468.

Gesell, R., *Am. J. Physiol.*, 1923, lxvi, 5.

Gesell, R., *Physiol. Rev.*, 1925, v, 551.

Gesell, R., and Hertzman, A. B., *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 298.
Haggard, H. W., and Henderson, Y., *J. Biol. Chem.*, 1920, xliii, 15.
Hastings, A. B., and Sendroy, J., Jr., *J. Biol. Chem.*, 1924, lxi, 695.
Henderson, Y., and Haggard, H. W., *J. Am. Med. Assn.*, 1922, lxxviii, 697.
Lundsgaard, C., *J. Exp. Med.*, 1919, xxx, 147.
Means, J. H., Bock, A. V., and Woodwell, M. N., *J. Exp. Med.*, 1921. xxxiii, 201.
Milroy, T. H., *J. Physiol.*, 1917, li, 259.
McGinty, D. A., and Gesell, R., *Am. J. Physiol.*, 1925, lxxv, 70.
Penfield, W. G., *Am. J. Physiol.*, 1919, xlviii, 121.
Peters, J. P., Bulger, H. A., and Eisenman, A. J., *J. Biol. Chem.*, 1923, lv, 687.
Robertson, O. H., and Bock, A. V., *J. Exp. Med.*, 1919, xxix, 139.
Tatum, A. L., *J. Biol. Chem.*, 1920, xli, 59.
Van Slyke, D. D., Austin, J. H., and Cullen, G. E., *J. Biol. Chem.*, 1922, liii, 277.
Van Slyke, D. D., and Stadie, W. C., *J. Biol. Chem.*, 1921, xlix, 1.
Van Slyke, D. D., Wu, H., and McLean, F. C., *J. Biol. Chem.*, 1923, lvi, 765.
Winterstein, H., *Arch. ges. Physiol.*, 1921, clxxxvii, 293.

A COMPARISON OF THE pH OF SERUM AND PLASMA OF DOG BLOOD.*

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(Received for publication, May 18, 1926.)

While the hemorrhage experiments reported in the previous paper were being carried out, it was thought advantageous to study serum instead of plasma in order to escape the slight trace of hemoglobin which is nearly always present in oxalated dog plasma. Serum is different from plasma in that fibrinogen which is present in the latter has been precipitated, removing protein from solution and probably causing other changes of unknown nature. The removal of fibrinogen may alter the pH of the blood or may affect the color of the indicator. Therefore, if the data obtained with serum are to be used in discussing the chemistry of plasma, it is important to determine whether differences may be observed between the pH of dog plasma and serum.

While such comparisons were being made, a paper was published by Hirsch (1924) in which evidence was presented that serum is more acid than plasma. Hirsch studied, with a spectrophotometer, the color change of an indicator in rabbit plasma before and after clotting. From a measurement of these color changes he concluded that there was a decrease of pH with clotting. For rabbit plasma he found an average difference of 0.09 pH between the clotted and unclotted samples. In a single experiment on dog plasma the difference was 0.24 pH.

In a later article, however, which was published after the experiments presented in this paper were completed, Hirsch (1925) showed that the color changes determined by the spectrophotometric method could not be directly converted into pH values.

* A brief report of this paper is given in *Proc. Soc. Exp. Biol. and Med.*, 1925, xxiii, 115.

This makes doubtful the accuracy of the data and conclusions reported in his previous paper.

Kugelmass (1925) while studying the mechanism of blood clotting determined the pH of mixtures of plasma and thrombin, and fibrinogen and thrombin before and after clotting. He found that in each case the pH was greater after clotting. He concludes that fibrinogen absorbs hydrogen ions in the process of coagulation. The initial systems used were all of a much greater hydrogen ion concentration than normal blood, being all below pH 7.0. Since Kugelmass has shown by experiments that the higher the initial C_H the greater will be the diminution in hydrogen ion concentration of the system due to coagulation, it might be possible that, at the normal pH range of blood, there would be no noticeable change to the alkaline side. In the absence of a detailed description of his experiments the question arises whether the analytical results are in error due to the precipitation of fibrin on the electrodes.

EXPERIMENTAL.

The pH of serum or plasma was determined in the present experiments using Cullen's (1922) colorimetric and the standard electrometric[1] methods. Two blood samples were drawn merely by shifting the rubber tube attached to the needle from one container to another. In some experiments the plasma sample was collected first and in others the serum sample. In the first two experiments the plasma was obtained from blood drawn over mercury into a tonometer containing dried potassium oxalate and sodium fluoride as recommended by Evans (1922); in the other experiments the blood was collected under oil using oxalate alone. The serum was obtained from clotted or defibrinated blood collected under oil. All specimens of blood were centrifuged under a layer of solidified paraffin.

Table I gives the results of the determinations on normal dog blood. They show that the pH was the same for both plasma and serum. The correction for the colorimetric pH which Cullen found to be 0.35 pH for dog plasma varied considerably. It was slightly greater for serum than for plasma, as stated by Cullen.

[1] Bennett, M. A., *J. Biol. Chem.*, 1926, lxix, 675.

TABLE I.

Comparison of the pH of Serum and Plasma.

Date.		Hemolysis.	Electrometric pH.	Colorimetric pH uncorrected.	Colorimetric pH correction.	Remarks.
1924						
Nov. 24.	Serum.	None.	7.39	7.85	0.46	First collection (clotted).
	Plasma.	Slight.	7.39	7.81	0 42	Second " (over mercury).
" 26.	Serum.	Very slight.	7.39	7.72	0.33	Second " (clotted).
	Plasma.	Moderate.	7.39	7.68	0.29	First " (over mercury).
			7.37	7.68	0.31	
Dec. 4.	Serum.	None.	7.28	7.75	0.47	" " (clotted).
	Plasma.	Slight.	7.31	7.70	0.39	Second " (under oil).
" 8.	Serum.	None.	7.30	7.73	0.43	First " (clotted).
			7.32	7.73	0.41	
	Plasma.	Slight.	7.31	7.73	0.42	Second " (under oil).
			7 30	7.73	0.43	
" 13.	Serum.	"	7.35	7.70	0.35	First " (defibrinated).
	Plasma.	Considerable.	7.34	7.63	0.30	Second " (under oil).
" 17.	Serum.	None.	7.35	7.75	0.40	" " (clotted).
			7.37	7.75	0.38	
	Plasma.	Slight.	7.35	7.73	0.38	First " (under oil).
			7.36	7.73	0.37	

SUMMARY.

A comparison was made of the pH of plasma and serum of blood from normal dogs. No difference was found.

BIBLIOGRAPHY.

Cullen, G. E., *J. Biol. Chem.*, 1922, lii, 501.
Evans, C. L., *J. Physiol.*, 1922, lvi, 146.
Hirsch, E. F., *J. Biol. Chem.*, 1924, lxi, 795.
Hirsch, E. F., *J. Biol. Chem.*, 1925, lxiii, 55.
Kugelmass, I. N., Colloid symposium monograph, New York, 1925, iii, 158.

A NOTE ON CULLEN'S COLORIMETRIC METHOD FOR THE DETERMINATION OF THE pH OF BLOOD PLASMA.*

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In studying the effect of hemorrhage upon the acid-base equilibrium in the blood of dogs, both Cullen's (1922) colorimetric method and the electrometric method were used for the determination of the hydrogen ion concentration of plasma and serum. The colorimetric method requires much less time and blood, and would therefore be preferable. But since this method requires, for dog blood, a large correction (0.35 pH) which at the time of the present experiments had not been studied under abnormal conditions, it was thought advisable to check Cullen's method by the electrometric method.

The correction is necessary to convert the colorimetric readings at 20°C. to the electrometric readings at 38°C. owing to variations in the pH of the solution and in the color of the indicator due to protein and salts, and the difference in temperature. As the composition of the blood varies considerably after hemorrhage, it was thought that the correction might not remain constant.

While these experiments were being carried out, Hastings and Sendroy (1924) reported that Cullen's correction disappeared if the colorimetric determinations were made at 38°C. instead of at 20°C. They state that the salt error and protein error are negligible in determinations carried out by Cullen's procedure; and the dilution itself does not influence the readings. Therefore the temperature correction is the only one of importance, and this, they state, disappears if the colorimetric readings are made at

* A brief report of this paper is given in *Proc. Soc. Exp. Biol. and Med.*, 1925, xxiii, 115.

38°C. In one of the present experiments, the readings were made at 38°C., but contrary to the observations of Hastings and Sendroy (1924) there was not a complete disappearance of the correction.

About the time that these observations were being made, Hastings, Neill, Morgan, and Binger (1924) reported that the colorimetric correction for human plasma in pneumonia was greater than the normal correction by 0.05 pH. Since then, Austin and Cullen (1925) and Cullen, Keeler, and Robinson (1925) have noted variations in the colorimetric correction and the inadequacy of the modification of Hastings and Sendroy (1924). Austin, Stadie, and Robinson (1925) stimulated by the data reported in the present paper, have studied the situation in detail, have reached similar conclusions, and have suggested a modification of the method.

EXPERIMENTAL.

The methods used in the present experiments were carried out as follows. Blood was drawn under oil, without stasis and without loss of CO_2, into a tube coated with sufficient potassium oxalate to make a 0.3 per cent solution when mixed with the blood. It was centrifuged in the tube which was completely filled with blood and stoppered, or overlaid with paraffin as suggested by Hastings and Sendroy (1924). At no time was the blood allowed to come into contact with air. The serum was obtained by allowing the blood to clot in the tube and then centrifuging in the same manner as the plasma. The pH of the plasma or serum was then determined by Cullen's colorimetric method and by the electrometric method, following the usual procedure. The colorimetric readings were made at 20°C. and some at 38°C., the electrometric determination was made at 38°C.

As shown by Table I the correction necessary to convert the colorimetric readings at 20°C. to electrometric readings at 38°C. did not stay constant. The correction varies from 0.35 to 0.42 in the normal sera studied. A variation of this type due to the differences of blood of different individuals would probably not invalidate conclusions drawn from colorimetric determinations. But a far more serious difficulty is evident when the variations in the correction for determinations on blood from an individual animal are examined. The correction was found to vary as much as from 0.24 to 0.41 when blood from one animal was studied from

TABLE I.

Effect of Hemorrhage on the Colorimetric pH Correction.

	Electrometric pH.	Colorimetric pH uncorrected.	Colorimetric pH correction.	Electrometric pH.	Colorimetric pH uncorrected.	Colorimetric pH correction.
	Dog 1. Plasma (first hemorrhage).			Dog 2. Plasma (first hemorrhage).		
Normal	7.31	7.66	0.35			
"	7.31	7.65	0.34	7.34	7.68	0.34
"	7.30	7.65	0.35	7.37	7.73	0.36
½ hr. after hemorrhage				7.29	7.65	0.36
4 hrs. " "	7.22	7.63	0.41		7.60	
24 " " "	7.37	7.61	0.24	7.45	7.71	0.26
3 days " "				7.40	7.66	0.26
10 " " "				7.35	7.56	0.21
	Dog 3. Serum (first hemorrhage).			Dog 2. Plasma (second hemorrhage).		
Normal	7.33	7.75	0.42			
½ hr. after hemorrhage	7.31	7.73	0.42	7.36	7.58	0.22
4 hrs. " "	7.26	7.75	0.49	7.25	7.55	0.30
24 " " "	7.38	7.75	0.37		7.67	
3-4 days " "	7.34	7.73	0.39	7.36	7.60	0.24
6 " " "	7.33	7.73	0.40			
46 " " "				7.26	7.58	0.32
	Dog 3. Serum (second hemorrhage).			Dog 4. Serum (first hemorrhage).		
Normal				7.34	7.70	0.36
½ hr. after hemorrhage	7.32	7.75	0.43			
4 hrs. " "	7.31	7.75	0.44	7.32	7.69	0.35
24 " " "	7.39	7.75	0.36	7.35	7.64	0.29
9-14 days after hemorrhage	7.34	7.70	0.36	7.34	7.75	0.41
21 " " "	7.33	7.70	0.37			
	Dog 3. Serum (third hemorrhage).			Dog 4. Serum (second hemorrhage).		
Normal						
½ hr. after hemorrhage						
4 hrs. " "	7.32	7.70	0.38	7.32	7.64	0.32
24 " " "	7.38	7.75	0.37	7.37	7.70	0.33

day to day. Curiously enough, the correction varied in such a way, that the variations of the colorimetric readings were less than those of the electrometric determinations and in some cases disappeared almost completely. The corrections for both serum and plasma varied in a corresponding manner, though the serum correction seemed to range higher than that for plasma.

TABLE II.

Effect of Hemorrhage and Gelatin Saline Injection on the Colorimetric pH Correction. Dog. 5, Serum.

	Electrometric pH.	Colorimetric pH uncorrected.	Colorimetric pH correction.
Normal	7.35	7.70	0.35
1 hr. after hemorrhage and 5 min. after injection of 6 per cent gelatin saline solution	7.26	7.60	0.34
4½ hrs. after injection of gelatin	7.44	7.78	0.34
24 " " " " "	7.31	7.65	0.34

TABLE III.

Corrections for Colorimetric pH Readings Made at 20° and 38°C.

	Electrometric pH.	Colorimetric pH uncorrected 20°C.	Colorimetric pH correction 20°C.	Colorimetric pH uncorrected 38°C.	Colorimetric pH correction 38°C.
Normal plasma	7.30	7.65	0.35	7.45	0.15
½ hr. after hemorrhage.	7.23	7.65	0.42	7.45	0.22
5 hrs. " "		7.65		7.45	
24 " " "		7.72		7.47	
48 " " "	7.34	7.74	0.40	7.52	0.18
Plasma	7.39	7.81	0.42	7.60	0.21
Serum	7.39	7.85	0.46	7.60	0.21
"		7.52		7.35	

About 4 hours after hemorrhage the correction had risen from 0.06 to 0.08 above the normal value. On the day following a hemorrhage, the correction was often from 0.05 to 0.09 below the normal value. Thereafter the value tended to rise slowly to a normal level.

No variation in the correction was observed in one experiment

in which gelatin saline solution was infused immediately after hemorrhage. (Table II).

In one experiment the colorimetric readings were made at 38°C., as well as at 20°C. (Table III). Although the corrections were not as large when the readings were made at 38°C., they did not disappear, and showed as great variation in the same general directions after hemorrhage as did the 20°C. correction. These results are at variance with those of Hastings and Sendroy (1924) who found the correction to disappear entirely.

The causes for the variations of the colorimetric correction are unknown. The great variation which occurs after hemorrhage might be attributed to differences in the concentration of fibrinogen in plasma (Foster and Whipple, 1922). Such an explanation fails when it is found that the correction changes in serum as well as in plasma. It is impossible with the information available at present, to know whether other factors causing or resulting from blood coagulation can influence the results. Marked changes occur in the concentration of serum proteins as well as the ratio of globulin to albumin after hemorrhage, but these changes offer no simple explanation for the observed variations (Marrack and Smith, 1924; Cullen, Keeler, and Robinson, 1925). The variations of the different salts of the serum (Kerr, 1926) seem too slight to warrant consideration.

SUMMARY.

Cullen's colorimetric method was found to be unreliable even for comparative studies after hemorrhage in dogs. The correction, necessary to convert the colorimetric readings at 20°C. to the electrometric pH at 38°C., was found to vary considerably. The correction for normal sera varied from 0.35 to 0.42. In an individual animal the correction varied from 0.24 to 0.41 after hemorrhage.

BIBLIOGRAPHY.

Austin, J. H., and Cullen, G. E., *Medicne*, 1925, iv, 275.

Austin, J. H., Stadie, W. C., and Robinson, H. W., *J. Biol. Chem.*, 1925, lxvi, 505.

Cullen, G. E., *J. Biol. Chem.*, 1922, lii, 501.

Cullen, G. E., Keeler, H. R., and Robinson, H. W., *J. Biol. Chem.*, 1925, lxvi, 301.
Foster, D. P., and Whipple, G. H., *Am. J. Physiol.*, 1922, lviii, 407.
Hastings, A. B., and Sendroy, J., Jr., *J. Biol. Chem.*, 1924, lxi, 695.
Hastings, A. B., Neill, J. M., Morgan, H. J., and Binger, C. A., *J. Clin. Inv.*, 1924, i, 25.
Kerr, S. E., *J. Biol. Chem.*, 1926, lxvii, 689, 721.
Marrack, J., and Smith, F. C., *Brit. J. Exp. Path.*, 1924, v, 13.